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VESICULAR-ARBUSCULAR MYCORRHIZAE ESTABLISHED WITH *GLOMUS FASCICULATUS* SPORES ISOLATED FROM THE FECES OF CRICETINE MICE

—FREDERICK M. ROTHWELL
Microbiologist
—COLEMAN HOLT
Research Wildlife Biologist
Northeastern Forest Experiment Station
Berea, Kentucky

Abstract. Cricetine mice were trapped on two revegetated surface-mined areas—one with a freshly seeded grass-legume cover and one with an early successional grass-forb cover. Chlamydospores of *Glomus fasciculatus* isolated from the feces of these animals produced representative endomycorrhizae with corn under greenhouse conditions.

INTRODUCTION

Mycorrhizae are essential to the growth and development of many plants. Most species of ectomycorrhizal fungi produce copious quantities of air-borne spores in nature. Many of these fungi have been cultured on laboratory media, and the dissemination of propagules for natural (Trappe 1962) or artificial (Palmer 1971; Marx and Bryan 1969) inoculation is thus readily accomplished. Conversely, the vesicular-arbuscular (VA) fungi are much more limited in their dispersal because of their subterranean sporulation, and spores are now thought to be distributed through host transplants and when they adhere to soil that is moved. A third means of dispersal considered is through the defecation of viable spores that have been ingested by small mammals and insects (Gerdemann and Trappe 1974). More recently Trappe and Maser (1976) demonstrated the viability of *Glomus macrocarpus* Tul. and Tul. spores that were removed from rodent feces and germinated in distilled water. In addition to spore germination as a requisite in defining the role of small mammals as likely vectors of the Endogonaceae, it is also necessary that excreted spores be capable of forming VA mycorrhizae. Accordingly, we report here on the isolation of spores of *Glomus fasciculatus* (Thaxter sensu Gerdemann) Gerdemann and Trappe from the feces of the eastern harvest mouse (*Reithrodontomys humulis* Audubon and Bachmann), the white-footed mouse (*Peromyscus leucopus* Rafinesque), and the prairie deer mouse (*P. maniculatus bairdii* Hoy and Kennicott), and the subsequent establishment of VA mycorrhizae—typical of those produced by soil-borne spores—with corn (*Zea mays* L.).

MATERIALS AND METHODS

The mice used in this study were trapped on two surface-mined areas in Pulaski County, Kentucky. One of these areas, the Woodall Branch plot, had a 3-month plant cover consisting of grasses and legumes. Vegetation on the other area, the Bolthouse Ridge plot, was in its second year of growth and consisted of introduced grasses and legumes as well as a variety of volunteer grasses and forbs. Trap lines extended out onto a reclaimed bench from just inside the adjacent residual stand of early successional shrub-forb growth. Trap stations were situated at prescribed locations along the lines. Two traps were set at each station with peanut butter and rolled oats as bait.

After mice were trapped, within 24 hours their gastrointestinal tracts were dissected intact from the animals. Fecal pellets removed from the lower tract were macerated in tap water and examined under a stereomicroscope. Specimens that contained spores were washed thoroughly in a nest of sieves having 90, 63, and 45 μm openings. Spores of *G. fasciculatus* that were retained by each of the sieves were selected and subsequently composited in 150 ml of distilled water. Fifty ml of a homogeneous suspension of the composite were added to each of three 4-in (10.16 cm) clay pots half-filled with sterile sand, then the pots were filled up with sterile sand and five grains of corn were planted in each. After the seeds germinated, 50 ml of a full nutrient growth solution were added on a weekly basis for 4 weeks. Distilled water was added throughout the growth period to maintain proper moisture conditions. At intervals of 18, 26, and 35 days after the seeds germinated, corn plants were removed from the pots and the root system washed free of sand. Small root segments were cut into a lactophenol-aniline blue solution, autoclaved for 10 minutes to stain the fungi, and examined microscopically for external and internal structures.

RESULTS AND DISCUSSION

Germination of spores (Fig. 1B) and appressoria produced by the developing mycelium were observed as early as 18 days. Thin-walled vesicles as well as inter- and intracellular mycelium were observed after 26 days; these were much more abundant 35 days after seed germination (Fig. 1 C–E). This study presents the initial evidence that *G. fasciculatus* spores that passed through the gastrointestinal tracts of small mammals are capable of producing representative VA mycorrhizae. Although the interval between the time feces are deposited by small mammals and when VA mycorrhizae develop in plants growing on mine spoil is unknown, climatic and edaphic factors undoubtedly influence the disintegration of feces and the subsequent distribution of fungal spores in the plant root zone. The absence of VA associates in plant roots examined from the recently seeded Woodall Branch plot would appear to reflect this.

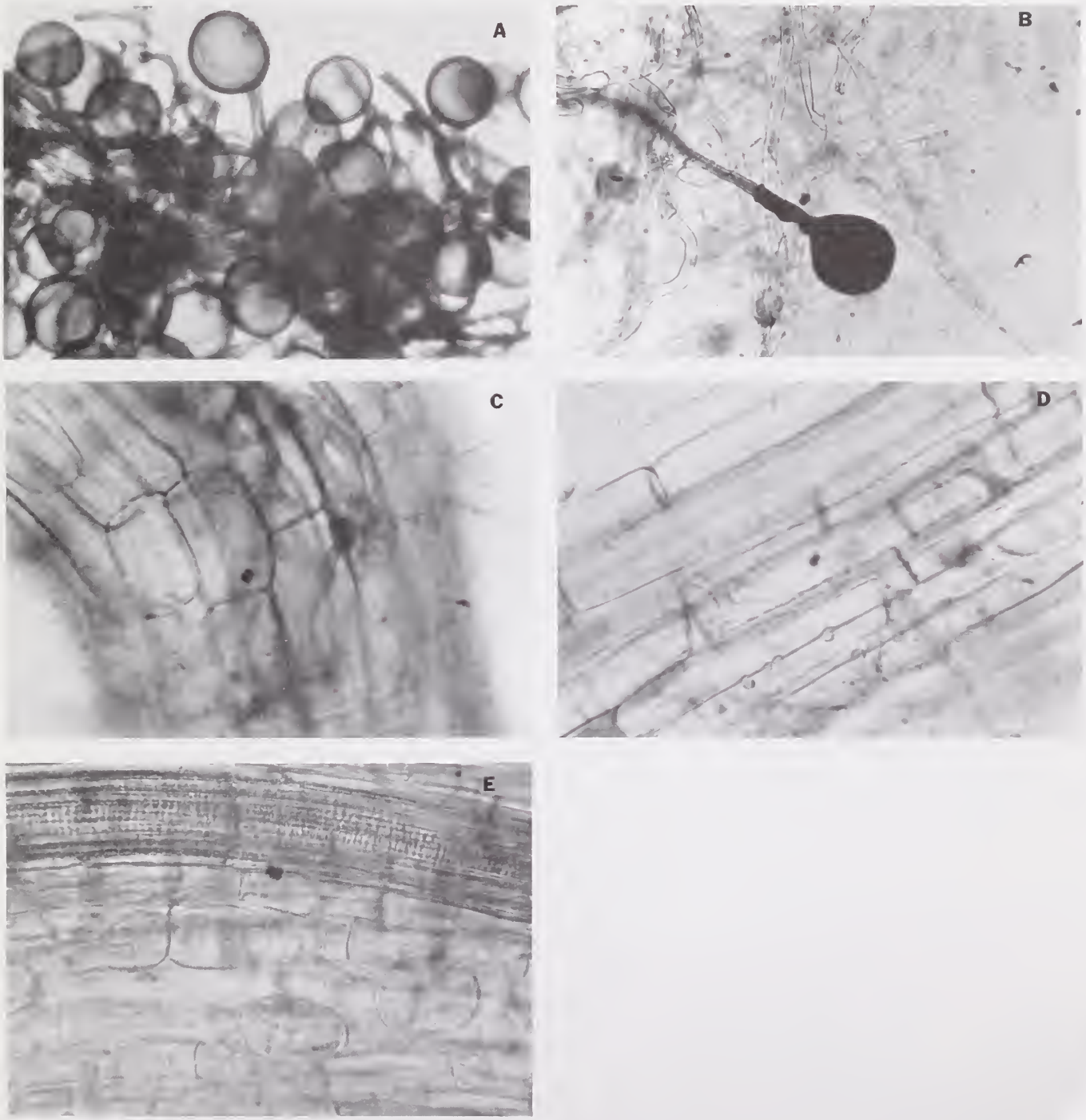
Data from trapping indicate that the prairie deer mouse, the white-footed mouse, and the eastern harvest mouse are early arrivals after mining. A specimen of the eastern harvest mouse was trapped on the Woodall Branch plot approximately 275 ft (84 m) from cover. Likewise, on nearby mine spoils with plant composition and age comparable to this sample plot, *Peromyscus* spp. have been trapped up to 392 ft (119.5 m) away from protective vegetative cover. In both cases the mice were apparently feeding on ungerminated grains from the reclamation seeding.

A random sampling of the vegetation from the plot on Bolthouse Ridge showed that plants of all species had VA mycorrhizae approximately 2 years after reclamation (Table 1). On other mined sites with early successional plants, volunteer species of blackberry and dewberry (*Rubus* spp. L.), sassafras (*Sassafras albidum* (Nutt.) Nees), smooth sumac (*Rhus glabra* L.), and winged sumac (*R. copallina* L.) have all shown VA infection. Thus, it would appear that as plant growth on freshly graded surface-mined areas increases to early-successional and finally late successional growth on older sites similar to those described by Daft and Hacskaylo (1976), the introduced and/or

Table 1.—VA mycorrhizal species from an early successional grass-forb trapping plot, collected in October 1977

Species	Common name
Lespedeza stipulacea Maxim.	Korean lespedeza
Aster pilosus Willd.	Narrow-leafed aster
Aster sp.	—
Trifolium repens forma lodigens Hort. ex. Gams.	Ladino clover
Iva ciliata Willd.	Sumpweed
Bidens polylepsis Blake	Stick-tight

Figure 1.—Stages in development of VA mycorrhizae in *Zea mays* inoculated with *Glomus fasciculatus* chlamydospores. Days indicate time interval following seed germination. A.—Representaive cluster of chlamydospores used as inoculum. B.—Single spore germination after 18 days. C-D.—Intracellular hyphae (coils) after 35 days. E.—Vesicle formation from intercellular mycelium after 35 days.



volunteer plant species show an increased prevalence of VA mycorrhizal fungi. Since VA associates are essential to the growth and development of many plant species, and inasmuch as small mammals are encountered in surprisingly large numbers on revegetated surface-mined areas, it is probable that these animals are active vectors in the colonization of the ecologically important endophytes on surface-mined areas.

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